Ecological Applications, 10(1), 2000, pp. 3–17

GAS EXCHANGE, LEAF NITROGEN, AND GROWTH EFFICIENCY OF *POPULUS TREMULOIDES* IN A CO_2-ENRICHED ATMOSPHERE

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Abstract. Predicting forest responses to rising atmospheric CO_2 will require an understanding of key feedbacks in the cycling of carbon and nitrogen between plants and soil microorganisms. We conducted a study for 2.5 growing seasons with *Populus tremuloides* grown under experimental atmospheric CO_2 and soil-N-availability treatments. Our objective was to integrate the combined influence of atmospheric CO_2 and soil-N availability on the flow of C and N in the plant–soil system and to relate these processes to the performance of this widespread and economically important tree species. Here we consider treatment effects on photosynthesis and canopy development and the efficiency with which this productive capacity is translated into aboveground, harvestable yield.

We grew six *P. tremuloides* genotypes at ambient (35 Pa) or elevated (70 Pa) CO_2 and in soil of low or high N mineralization rate at the University of Michigan Biological Station, Pellston, Michigan, USA (45°35′ N, 84°42′ W). In the second year of growth, net CO_2 assimilation rate was significantly higher in elevated-CO_2 compared to ambient-CO_2 plants in both soil-N treatments, and we found little evidence for photosynthetic acclimation to high CO_2. In the third year, however, elevated-CO_2 plants in low-N soil had reduced photosynthetic capacity compared to ambient-CO_2, low-N plants. Plants in high-N soil showed the opposite response, with elevated-CO_2 plants having higher photosynthetic capacity than ambient-CO_2 plants. Net CO_2 assimilation rate was linearly related to leaf N concentration (log : log scale), with identical slopes but different intercepts in the two CO_2 treatments, indicating differences in photosynthetic N-use efficiency. Elevated CO_2 increased tissue dark respiration in high-N soil (+22%) but had no significant effect in low-N soil (+9%). There were no CO_2 effects on stomatal conductance. At the final harvest, stem biomass and total leaf area increased significantly due to CO_2 enrichment in high-N but not in low-N soil. Treatment effects on wood production were largely attributable to changes in leaf area, with no significant effects on growth efficiency. We conclude that harvest intervals for *P. tremuloides* on fertile sites will shorten with rising atmospheric CO_2, but that tree size at canopy closure may be unaffected.

Key words: carbon dioxide, elevated; feedbacks in the plant–soil system; forest responses to rising atmospheric CO_2; gas exchange, plant–soil system; global climate change, ecological effects; growth efficiency; leaf nitrogen; Michigan (USA); photosynthesis; Populus tremuloides; *respiration; soil-N availability.*

INTRODUCTION

Elevated atmospheric CO_2 and plant–soil interactions

The common limitation of nitrogen (N) for plant growth and carbon (C) for microbial growth in soil results in close coupling of the plant and soil C and N cycles (Pastor and Post 1988). At a fundamental level, the response of these biogeochemical cycles to rising atmospheric CO_2 will be controlled by the growth response of plants and the extent to which changes in above- and belowground litter inputs alter the composition and function of microbial communities in soil. To further our understanding of plant–soil interactions with rising atmospheric CO_2 and hence our ability to predict changes in ecosystem function, greater attention must be paid to experiments focusing on key feedbacks

Manuscript received 16 October 1998; revised 12 April 1999; accepted 22 April 1999; final version received 20 May 1999. For reprints of this Invited Feature, see Footnote 1, p. 1.
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in the cycling of C and N between plants and soil microorganisms (Curtis et al. 1994). These feedbacks occur within a complex chain of assimilation, transport, transformation, and release of these elements and require approaches that integrate physiological and growth processes operating in different spatial domains and time scales.

Soil microorganisms represent important sinks as well as sources of N (Vitousek and Matson 1984, Zak et al. 1990), and they are the main regulators of C dynamics within the soil (McGill et al. 1986). Increased fine-root and leaf production, and altered tissue biochemistry, could potentially alter microbial growth in soil, and thus influence the flow of N from soil microorganisms to plant roots. Although the interaction between plants and soil microorganisms has important consequences for C sequestration in terrestrial ecosystems, there are fundamental gaps in our knowledge of the mechanisms by which rising atmospheric CO_2 will alter the exchange of C and N between plants and soil microorganisms. For example, we understand relatively little regarding how atmospheric CO_2 will modify the production and timing of root C inputs to soil and how this input of substrate will influence the composition and function of microbial communities in soil. Moreover, it is likely that differences in soil-N availability will modify plant growth response to atmospheric CO_2, but we have little information about this potential feedback on the flow of C and N through the soil food web. Some experimental evidence suggests that C and N cycling will slow under elevated CO_2 due to the production of plant litter that will enhance rates of microbial immobilization (Diaz et al. 1993, Berntson and Bazzaz 1997), whereas others have observed increased rates of C and N transformations (Zak et al. 1993, Hungate et al. 1997). Whether rising atmospheric CO_2 will increase, decrease, or not influence rates of C and N cycling in soil has important global implications for C sequestration in terrestrial ecosystems.

In 1993 we initiated an experiment to study the combined influence of atmospheric CO_2 and soil-N availability on the cycling of C and N between plant roots and soil microorganisms. We grew softwood cuttings of *Populus tremuloides* Michx. under experimental atmospheric-CO_2 and soil-N-availability treatments for three growing seasons in large open-top chambers. Our experiment was designed to test the broad hypothesis that there is long-term positive feedback between CO_2 assimilation at elevated CO_2, root growth, microbial populations, and soil-N availability (Zak et al. 1993). More specifically, we hypothesized that plant C assimilation will increase in an elevated-CO_2 atmosphere, even under conditions of low soil-N availability. Moreover, we reasoned that this increase will be maintained over time by greater C translocation belowground and increased N acquisition. The latter will be driven by larger fine-root production and greater mycorrhizal infection under CO_2 enrichment, resulting in greater soil exploration. Short-term soil-C availability could increase in response to greater rates of fine-root production (or turnover), and greater soil-C availability would elicit an increase in soil microbial biomass. We also predicted that net N mineralization would increase as a result of either an increase in the turnover of microbial N (via increased protozoan grazing) or through greater organic-matter mineralization by a larger microbial population.

Carbon dioxide assimilation at high CO_2

Regardless of the physiological links between plants and soil microorganisms, the first stage in signal transduction from atmospheric C to biogeochemical processes in soil is the assimilation of CO_2 by plants. There is now a sizable body of literature detailing the gas-exchange responses of woody plants to CO_2 enrichment, with some convergence in estimates of the overall direction and magnitude of CO_2 effects on key physiological processes and allocational patterns in young ($<$5 yr) plants. Under optimal growth conditions (i.e., given ample water, nutrients, and light) it is clear that leaf-level light-saturated net CO_2 assimilation (hereafter called ''*A*'') shows a positive, sustained increase under twice-ambient CO_2, with estimates from quantitative reviews ranging from +44% (Gunderson and Wullschleger 1994) to +66% (Norby et al. 1999). A sustained increase in the assimilatory capacity of leaves is central to many predictions of greater forest productivity with rising CO_2 (Reynolds et al. 1996). Although the effect of elevated CO_2 on *A* is probably the best understood plant CO_2 response at a mechanistic level, our understanding of the environmental factors causing down-regulation, or acclimation of *A* at high CO_2 (A_{accl}, sensu Gunderson and Wullschleger 1994) remains incomplete. Under some conditions root restriction elicits a strong A_{accl} response (Thomas and Strain 1991) suggesting an important role for source : sink dynamics, but this result is far from universal (McConnaughay et al. 1993). Gunderson and Wullschleger (1994) estimated a mean 21% A_{accl} across 20 studies with some indication of greater A_{accl} under low nutrients, a conclusion supported by Curtis (1996). In a more recent review, Curtis and Wang (1998) found no evidence for systematic A_{accl} by trees, except for those grown in pots $<$0.5 L.

A promising line of research that may improve our ability to predict A_{accl} under different environmental conditions builds on the well-recognized positive relationship between *A* and leaf N concentration (Field and Mooney 1986) and on the inherent tradeoffs between C and N allocation to leaf structural or defense functions on the one hand and to assimilatory capacity on the other (Lambers and Poorter 1992). The magnitude of the CO_2 response by *A,* and the probability of A_{accl}, may be largely a function of N and structural

biomass allocation within the leaf (Luo et al. 1994) and may follow predictable patterns across biomes or plant functional groups (Peterson et al. 1999). Framing the consideration of elevated-CO_2 effects on *A* within the context of leaf N and specific leaf area (SLA) has the additional benefit of allowing one to relate photosynthetic responses following experimental CO_2 manipulations to broader patterns of plant adaptation and functioning within natural ecosystems (e.g., Reich et al. 1997).

Biomass also is consistently, positively affected by elevated CO_2 under optimal conditions, but as growth conditions diverge from optimal our certainty in the average CO_2 effect size is considerably less, as evidenced by markedly different conclusions reached in recent reviews. For example, while McGuire et al. (1995) and Curtis and Wang (1998) estimated a halving of the CO_2 effect on biomass accumulation due to growth under low-nutrient conditions, Idso and Idso (1994) and Wullschleger et al. (1995) concluded that there was no effect of nutrients on the magnitude of the CO_2 response (i.e., it was equivalent to that under optimal conditions). These different conclusions reflect both different quantitative review methods and methodological differences among the primary studies considered by the reviewers, such as the presence of interacting stress variables, length of CO_2 exposure, pot size, or type of exposure facility, which can affect the CO_2 response independently of soil nutrient status (Curtis 1996). Norby (1996) suggested that biomass gain per se may not be the best measure of woody-plant growth response to elevated CO_2 precisely because it is so sensitive to environmental and cultural conditions. Short-term effects on development are amplified over time in young plants so that biomass gain during early growth may be poorly related to CO_2 effects occuring later in stand development. As an alternative he proposed the canopy productivity index, or growth efficiency (*E*, wood production per unit leaf area per unit time) sensu Waring (1983) as a measure better tied conceptually to the control of productivity in trees. For example, as stand leaf-area index (LAI) increases, with consequent increases in competition for light, *E* typically declines in a manner reflective of species-specific adaptational characteristics and of site conditions. Factors allowing the maintenance of high *E* as LAI increases, such as shade tolerance or nutrient addition, lead to sustained increases in stand productivity during canopy closure (Waring 1983). Because CO_2 enrichment can affect canopy development itself (e.g., Reekie and Bazzaz 1989, Ceulemans et al. 1995, Curtis et al. 1995) *E* may be a more conservative indicator of overall performance and a better predictor of long-term behavior than is biomass gain. Indeed, among seven elevated-CO_2 experiments in which *E* could be calculated, the coefficient of variation (CV) for mean percentage increase in aboveground dry mass due to elevated CO_2 was 75% compared to a 24% CV for the mean CO_2 effect on *E* (Norby 1996).

In this paper, we report the effects of elevated CO_2 on photosynthetic C assimilation and growth of *P. tremuloides*, the first step in the movement of C from the atmosphere to the biogeochemical cycling of C and N by soil microorganisms. Our objectives were to characterize the pattern of C gain over time as influenced by CO_2 and soil-N availability and to put these observations into the larger picture of tree growth dynamics and long-term stand productivity. Our null hypothesis was that C assimilation and growth would show a continued, positive response to CO_2 enrichment, even in low-N soil We reasoned that greater belowground growth under elevated CO_2 would increase plant N acquisition, thus maintaining greater rates of C acquisition at elevated compared to ambient CO_2 (Zak et al. 1993).

METHODS

Our experiment was conducted at the University of Michigan Biological Station, Pellston, Michigan, USA, 45°35′ N, 84°42′ W. An array of 20 open-bottom root boxes (3.3 × 3.3 × 0.4 m) were placed in an open field in October 1993 and filled with soil. The root boxes rested on soil surface whose A and E horizons had previously been removed. Each root box was lined with 1.3-cm-thick styrofoam insulation and plastic sheeting and contained eight minirhizotron tubes (Pregitzer et al. 2000). Two soil-N-availability treatments were established by filling half of the root boxes with the A horizon of a Kalkaska series topsoil (high-N treatment), a common soil type in northern lower Michigan, and the remaining boxes with a mixture of 20% Kalkaska A horizon and 80% Rubicon C horizon sand (low-N treatment). Five centimeters of 100% Rubicon sand were placed over the soil surface of each root box to equalize surface albedo. Net nitrogen mineralization was significantly higher in the high-N soil (318 ng $N \cdot g^{-1} \cdot d^{-1}$) than in the low-N soil (62 ng $N \cdot g^{-1} \cdot d^{-1}$, Zak et al. 2000*b*). These N mineralization rates are well within the range normally encountered in soils of this region and where aspen would be expected to establish following disturbance (Zak et al. 2000*a*). Other physical and chemical properties of the two soils are presented in Table 1. Soil texture was determine using the hydrometer method. Ceramic-plate pressure membranes were used to determine soil water content at −0.03 and −1.50 MPa. Total C and N were measured using an NC2500 Elantech elemental analyzer (CE Elantech, Lakewood, New Jersey, USA). The Bray P1 method (Kuo 1996) was used to extract PO_4^{3-}, and P concentrations were determined with an Alpkem RFA 300 autoanalyzer (Alpkem, Wilsonville, Oregon, USA). Soil pH was measured in a 1:1 soil-deionized water paste with a glass electrode.

Open-top chambers (3 × 2.3 m, Heagle et al. 1989)

TABLE 1. Physical and chemical properties of low- and high-N soils.

Soil properties	Low-N soil		High-N soil	
	Mean	1 SE	Mean	1 SE
Physical properties				
Sand (%)	93.5[a]	0.30	72.4[b]	2.14
Silt (%)	4.0[a]	0.47	17.2[b]	1.43
Clay (%)	2.5[a]	0.50	10.1[b]	1.02
Gravimetric water content, ω				
−0.03 MPa	0.031[a]	0.002	0.115[b]	0.010
−1.50 MPa	0.017[a]	0.01	0.062[b]	0.005
Available water content	0.014[a]	0.002	0.053[b]	0.012
Chemical properties				
Total C (mg C/kg soil)	3559[a]	454.7	12 489[b]	2273.8
Total N (mg N/kg soil)	260[a]	21.3	996[b]	169.9
C:N	13.7[a]	0.23	12.5[a]	1.77
Extractable P (mg P/kg soil)	13.7[a]	0.68	10.4[b]	1.10
pH	6.74[a]	0.177	6.08[b]	0.127

Notes: Six soil cores (5-cm diameter) were collected to a depth of 10 cm in each open-bottom root box. Cores were composited on a chamber basis and homogenized. All analyses were conducted on the composite sample from each open-bottom root box; $n = 10$ replicate root boxes per soil-N availability treatment. Within a row, means with the same lowercase superscript letter are not significantly different at $P < 0.05$.

were used to manipulate atmospheric-CO_2 partial pressure. Ten chambers received additional CO_2 (elevated-CO_2 treatment) and 10 chambers received no additional CO_2 (ambient-CO_2 treatment). Carbon dioxide treatments were randomized across soil-N availability treatments within five replicate blocks. Carbon dioxide partial pressure was increased by dispensing 100% CO_2 into an input blower via manual flowmeters with the atmosphere inside all elevated-CO_2 chambers and one ambient-CO_2 chamber monitored continuously using an infrared gas analyzer. Carbon dioxide treatments were maintained 24 hr/d for all days during which green leaves were present in the chambers, but CO_2 fumigation was terminated following leaf senescence in 1994 and 1995. Daytime (0701–1900) elevated-CO_2 treatment was 70.7 ± 0.06 Pa (mean of daily averages across all chambers and days ± 1 SE) and nighttime (1901–0700) elevated-CO_2 treatment was 73.2 ± 0.14 Pa. The ambient-CO_2 treatment was 35.7 ± 0.06 Pa (day) and 38.32 ± 0.09 Pa (night). Light and temperature were continuously monitored every 10 min inside and outside chambers during the growing season. Daytime temperatures were 1.34 ± 0.24°C (mean ± 1 SE) higher inside than outside chambers across the three growing seasons. The chamber plastic transmitted 81% of ambient PAR (photosynthetically active radiation).

On 7 June 1994 two individuals from each of six locally derived trembling aspen (*Populus tremuloides* Michx.) genotypes were transplanted into each root box (=12 trees per chamber) and CO_2 treatments begun. Genotype was therefore a subplot within the CO_2 × soil-N whole plot, yielding a split-plot randomized complete-block design. The genotypes were selected based on field observations of patterns of autumnal leaf senescence. Three genotypes (42E, 51E, and 61E) typically dropped their leaves in mid-October (early leaf-drop phenotype) and three genotypes (1L, 2L, and 8L) typically dropped their leaves in early November (late leaf-drop phenotype). By selecting these genotypes we hoped to encompass the variation present locally in a phenological trait that might influence the CO_2 response.

Individuals were arranged in two concentric circles within each chamber—an outer circle of eight trees and an inner circle of four trees. During June and July 1994 chambers were irrigated daily with 7–10 L water/m^2 soil surface, except on rainy or cloudy, cool days. Plants relied on natural precipitation from August 1994 until the beginning of the harvest in July 1996. In September 1995 all chambers were enlarged to 3.4 m in height to accommodate tree growth. During the following winter 5% of each tree's total aboveground wood volume was removed by pruning a portion of the terminal shoot. This was done to ensure containment of all trees within the open-top chambers until the end of the experiment.

Gas exchange

Leaf net CO_2 assimilation and stomatal conductance (g_s) were measured on 12 August 1995, and on 6 June and 16 July 1996 using a LI-COR LI-6400 portable photosynthesis system (LI-COR, Lincoln, Nebraska). Leaf temperature was maintained at 26°–30°C and saturating light intensity (1800–2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR) supplied with a cuvette illuminator. Gas-exchange measurements were performed between 1000 and 1600 on young, fully expanded leaves that had developed in full sun. CO_2 enriched air was supplied to the LI-6400 for measurements above ambient-CO_2 partial pressure ($p(CO_2)$).

Both A and g_s were measured at growth $p(CO_2)$ and at internal leaf $p(CO_2)$ (C_i) of 27 and 56 Pa. These C_i

values were representative of full-sun leaves from ambient- and elevated-CO_2 treatments, respectively, and allowed us to evaluate photosynthetic capacity independent of environmental effects on g_s. Leaves were first exposed to their growth p(CO_2) until steady-state gas exchange was observed, then measurements made at 27 Pa C_i, followed by 56 Pa C_i. At each measurement p(CO_2), leaves were allowed to equilibrate for at least 4 min before measurements of A (leaf-level light-saturated net CO_2 assimilation) or g_s (stomatal conductance) were made. Following each measurement, 2.6-cm^2 leaf discs were collected from the sampled leaf, frozen on dry ice, and later lyophilized to dryness. These samples were used for calculation of SLA (specific leaf area) and of leaf N on a mass (N_m) or an area (N_a) basis. Tissue N was measured with a CE Elantech CN 1200 elemental analyzer. Leaf discs were also collected from mature, high-light-grown leaves, embedded in paraffin, and 10-μm sections examined using light microscopy for determination of leaf thickness.

Leaf respiration was measured on shaded leaves during the day once in 1995 (3 July, blocks 2–5), twice in 1996 (5 June and 9 July, blocks 2–5), and once on shaded leaves at night in 1996 (5 June, blocks 1–5). All leaf-respiration measurements were made at 28°C and at growth p(CO_2) using the LI-6400. In 1995 only genotypes 1L, 51E, and 61E were measured while in 1996 all genotypes were measured. Individuals were selected at random within a chamber, resulting in a partial repeated measure of individuals although branches sampled were always different. Stem respiration was measured once in 1996 (30 June–5 July, blocks 3–5, genotypes 1L and 61E) using the LI-6400 fitted with a custom branch cuvette; surface temperatures varied between 26° and 30°C and measurements were made at growth p(CO_2).

Growth measurements

In 1994 the total leaf area per plant (LA) was calculated by summing the area of individual leaves (LA_i), which was estimated by

$$\mathrm{LA}_i = x_l \times (L_\mathrm{l} \times L_\mathrm{w}) + y_l \quad (1)$$

where L_l was leaf length, L_w was leaf width, and x_l and y_l were genotype-specific regression coefficients obtained from destructive harvest of non-experimental plants. In 1995 LA was calculated by summing the leaf area of individual branches (LA_b), estimated by

$$\mathrm{LA}_\mathrm{b} = w_b \times B_\mathrm{d} + x_b \times B_\mathrm{l} + y_b \times (B_\mathrm{l} \times B_\mathrm{d}) + z_b, \quad (2)$$

where B_l was branch length, B_d was branch basal diameter, and w_b, x_b, y_b, and z_b were genotype-specific regression coefficients obtained from measuring L_l and L_w on all leaves from a subset of experimental branches and applying Eq. 1. Stem volume was estimated from B_l and B_d assuming a conical stem geometry.

Growth efficiency was calculated both on the basis of estimated yearly stem-volume increase (aboveground volume growth efficiency, E_v) and on the basis of harvested stem dry mass (E_dm). All growth-efficiency calculations were made on the basis of growth per chamber (as opposed to growth per plant) of the four plants growing in the center of the chamber, thereby eliminating the influence of side light, using the following expressions:

$$E_\mathrm{v} = \frac{V_n - V_{n-1}}{d_n \times \mathrm{LA}_n} \quad (3)$$

$$E_\mathrm{dm} = \frac{S_\mathrm{dm}}{401 \times \sum_{n=1994}^{1996} \mathrm{LA}_n} \quad (4)$$

where V_n was estimated stem volume in year n, LA_n was estimated leaf area in year n, d_n was number of days since leaf out in year n, and S_dm was harvested stem dry mass. There were 401 d of leaf exposure during the entire study ($=\Sigma\, d_n$).

Plants were destructively harvested beginning 8 July 1996. Leaves were separated into three canopy-position classes based on their height above the soil surface: $<$1 m, 1–2 m, and $>$2 m for the high-N treatment, and $<$0.5 m, 0.5–1 m, and $>$1 m for the low-N treatment. Total leaf fresh mass per tree was obtained for each position class, the leaves were composited, and a subsample taken for leaf-area measurement (LI-COR LI-3000 leaf-area meter). This subsample was dried at 65°C and used for fresh mass : dry mass and mass : leaf area conversions for each tree. Leaf tissue C and N concentrations were determined from these subsamples using a CE Elantech CN 1200 elemental analyzer. Stem tissue was separated at each year's terminal bud scale scar, yielding first-year wood (produced in 1994, secondary growth in 1995 and 1996), second-year wood (produced in 1995, secondary growth in 1996), and third-year wood (produced in 1996). One ~10-cm subsample was cut from each age class and air dried for density determination. Stem volume at harvest for each individual was determined from stem age-class dry mass and stem age-class density. Zak et al. (2000*a*) and Pregitzer et al. (2000) provide details regarding the belowground harvest of this experiment.

Carbohydrate analysis

Leaf discs (2.6 cm^2) were excised between 1400 and 1500 on 3 July 1996 from the youngest fully expanded leaf on a branch exposed to full sun, immediately frozen in liquid nitrogen, and stored at −80°C until lyophilized. Powdered, lyophilized samples were extracted with 80% ethanol at 80°C, the supernatant evaporated to dryness, and then redisolved in H_2O + polyvinylpolypyrrolidone. Soluble carbohydrates were analyzed enzymatically using a modification of the procedure of Jones et al. (1977). The recovery rate was 95%. The ethanol-extracted tissue pellet was suspended

TABLE 2. Gas exchange of *Populus tremuloides* grown in low- or high-N soil and at ambient or elevated CO_2.

Date	Parameter	Low-N soil				High-N soil			
		Ambient CO_2		Elevated CO_2		Ambient CO_2		Elevated CO_2	
		Mean	1 SE	Mean	1 SE	Mean	1 SE	Mean	1 SE
August 1995	A_{gr}	21.0^{c}	1.4	26.4^{ab}	1.1	20.9^{bc}	1.9	32.6^{a}	2.1
	g_{sgr}	0.64^{a}	0.07	0.44^{a}	0.06	0.55^{a}	0.11	0.46^{a}	0.06
	A_{27}	21.5^{ab}	1.1	17.5^{c}	0.35	23.4^{a}	0.6	20.2^{bc}	1.0
	g_{s27}	0.65^{a}	0.06	0.41^{a}	0.04	0.54^{a}	0.12	0.42^{a}	0.07
	A_{56}	30.8^{ab}	1.2	26.5^{b}	1.1	34.8^{a}	0.9	33.7^{a}	2.3
	g_{s56}	0.54^{a}	0.06	0.43^{a}	0.05	0.49^{a}	0.12	0.46^{a}	0.08
July 1996	A_{gr}	9.1^{b}	1.0	12.1^{b}	1.6	9.2^{b}	0.8	21.0^{a}	1.9
	g_{sgr}	0.15^{a}	0.04	0.11^{a}	0.03	0.13^{a}	0.02	0.16^{a}	0.04
	A_{27}	11.9^{a}	0.8	6.7^{b}	0.7	12.9^{a}	1.5	15.1^{a}	1.2
	g_{s27}	0.15^{a}	0.04	0.10^{a}	0.02	0.13^{a}	0.02	0.13^{a}	0.04
	A_{56}	18.2^{b}	0.6	13.7^{c}	1.0	20.5^{b}	1.1	24.6^{a}	1.3
	g_{s56}	0.14^{a}	0.03	0.11^{a}	0.03	0.11^{a}	0.02	0.15^{a}	0.04

Notes: Measurements of net CO_2 assimilation (A) and stomatal conductance (g_s) were made at growth p(CO_2) (A_{gr}, g_{sgr}) and at internal p(CO_2) of 27 Pa (A_{27}, g_{s27}) and 56 (A_{56}, g_{s56}) Pa, respectively [p(CO_2) partial pressure]; $n = 5$ replicate chambers per CO_2 and soil-N availability treatment. Within a row, means with the same lowercase superscript letters are not significantly different at $P < 0.05$.

in 0.2 mol/L KOH, boiled for 20 min, and brought to pH 7.0 with 1.0 mol/L CH_3COOH. Amyloglucosidase (EC [Enzyme Classification] 3.2.1.3) was added to the resuspended pellet and incubated for 1 h at 55°C. Starch concentration was determined as glucose equivalents using the same procedure as for soluble sugars. Starch recovery was 93%.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) for a split-plot factorial block design where main effects (CO_2, and soil fertility) and $CO_2 \times$ soil fertility interaction were tested over the $CO_2 \times$ fertility nested within block mean square. The effect of genotype and all treatment interactions with genotype were tested over the error mean square. Growth efficiency, which was expressed on a per chamber basis, was analyzed as a two-way factorial ANOVA with five replicate blocks. Comparison among CO_2 and fertility treatment means was by least significant difference for a priori comparisons (between CO_2 treatments within a fertility treatment), and by minimum significant difference for all a posteriori comparisons (Sokal and Rohlf 1981).

The overall effect of CO_2 on respiration across years and tissue types was evaluated by calculating the 95% confidence interval around the mean CO_2 effect ($\overline{L}$), where

$$\overline{L} = \frac{\sum_{i=1}^{k} w_i L_i}{\sum_{i=1}^{k} w_i} \tag{5}$$

and $L_i = \log(\overline{X}_{ei}/\overline{X}_{ai})$, the log-transformed ratio of elevated ($\overline{X}_{ei}$) to ambient ($\overline{X}_{ai}$) CO_2 treatment means of the ith set of measurements, and w_i is the reciprocal of the total variance of L_i (Hedges et al. 1999). This is, therefore, a mean estimate weighted according to the precision (standard error) of the k separate studies and is typical for meta-analyses of this kind (Cooper and Hedges 1994).

The effect of CO_2 enrichment on the relationship between A and leaf N was evaluated using the linear model:

$$\log(A)_i = \beta_0 + \beta_1 \log(\text{leaf N})_i + \beta_2 X_i + \beta_3 \log(\text{leaf N})_i X_i + \varepsilon_i \tag{6}$$

where β_0 is the centered y intercept for ambient CO_2, β_1 is the slope for the ambient-CO_2 treatment, β_2 and β_3 are the change in the centered y intercept and slope, respectively, due to CO_2 enrichment, X_i is a dummy variable coded 0 for ambient CO_2 and 1 for elevated CO_2, and ε_i is residual error (Peterson et al. 1999). Significance of coefficients was determined by least-squares regression.

RESULTS

Gas exchange

Net CO_2 assimilation varied both among treatments and across years (Table 2). In mid-August 1995, A at growth p(CO_2) (A_{gr}) increased significantly in both soil N treatments (+56% and +26% in high- and low-N soil, respectively), but there was no effect of soil-N availability within CO_2 treatments. High-CO_2-grown plants in both soil N treatments had reduced photosynthetic capacity at 27 Pa C_i relative to ambient-CO_2-grown plants, but no reduction in capacity at 56 Pa C_i. There was no CO_2 or soil-N availability effect on g_s under any measurement C_i (Table 2).

By mid-July 1996 this picture had changed substantially (Table 2). Both A and g_s were significantly lower

TABLE 3. Analysis of variance of leaf area and stem dry mass (S_{dm}) per plant at the final harvest and for net CO_2 assimilation at growth p(CO_2) (A_{gr}) measured in July 1996.

Source of variation	df	Leaf area		S_{dm}		A_{gr}	
		MS	F	MS	F	MS	F
CO_2	1	5.8	10.4**	317 969	28.3***	1320	50.8***
Soil N	1	165.0	297.5***	7 437 212	663.2***	490	18.9**
CO_2 × Soil N	1	5.9	10.6**	219 824	19.6***	461	17.7**
Block	4	0.7	0.9	69 538	2.5	111	7.1***
Block (CO_2 × Soil N)	12	0.6		11 215		26	
Genotype	5	6.6	8.0***	324 596	11.6***	20	1.3
CO_2 × Genotype	5	1.1	1.3	19 532	0.7	7.2	0.5
Genotype × Soil N	5	4.3	5.3***	194 822	6.9***	9.0	0.5
CO_2 × Soil N × Genotype	5	0.5	0.6	19 732	0.7	5.9	0.4
Error	80	0.6		28 062		15.7	

Notes: Sources of variation were CO_2 treatment (ambient or elevated), soil-N availability (low or high), and genotype (six genotypes total). For A_{gr}, block df = 3.
** $P < 0.01$, *** $P < 0.001$.

in 1996 than in 1995, and the effect of CO_2 enrichment on A had diverged between the two soil-N-availability treatments. In high-N soil, A_{gr} was 128% greater in elevated-compared to ambient-CO_2-grown plants, while there was no significant CO_2 effect in low-N soil. This CO_2 × soil-N interaction also was reflected in the measurements of A at constant C_i. In low-N soil, elevated-CO_2-grown plants had significantly lower A at both measurement C_i's than did ambient-CO_2-grown plants. In high-N soil, however, A in elevated-CO_2-grown plants was equal to that in ambient-CO_2-grown plants when measured at 27 Pa CO_2, and greater than that in ambient-CO_2-grown plants when measured at 56 Pa CO_2. As in 1995, there were no treatment effects on g_s. In July 1996 we found no evidence for differential photosynthetic capacity among genotypes or in genotypic responses to the CO_2 or soil N treatments (Table 3).

Treatment effects on dark respiration were somewhat variable, with their magnitude changing over time, and between leaf and stem tissue (Table 4). In general, elevated-CO_2-grown plants had higher respiration rates than did ambient-CO_2-grown plants, the single exception being for stem respiration in plants from low-N soil. Because of low sample sizes, however, none of the individual CO_2 responses was statistically significant. To gain an estimate of the overall effect of CO_2 on aboveground respiration we combined these individual data sets, weighting each by their precision. The resulting mean CO_2 effect size suggests a greater positive respiratory response to CO_2 in high-N soil than in low-N soil, the latter being not significantly different from zero (95% CI overlaps zero). These confidence limits have some uncertainty associated with them since there was at least partial non-independence among individual data sets. However, applying a more conservative Type I error probability of 1% does not alter the result (CI of −2% to +22% for the low-N mean and 5% to 34% for the high-N mean).

Net assimilation in 1996, expressed on a tissue mass basis, showed a strong positive relationship with N_m (Fig 1). Plotted on a log : log scale this relationship was linear, with elevated- and ambient-CO_2 treatments having identical slopes of 1.39 at growth p(CO_2) and 1.31

TABLE 4. Dark respiration in leaves and stems of *Populus tremuloides* grown in low- or high-N soil and at ambient or elevated CO_2.

Tissue sample	Low-N soil			High-N soil		
	Ambient CO_2 ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Elevated CO_2 ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Change (%)†	Ambient CO_2 ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Elevated CO_2 ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Change (%)†
Leaf, 1995 day	2.49 (0.22)	3.12 (0.12)	+25.3	3.02 (0.28)	3.48 (0.27)	+15.2
Leaf, 1996a day	2.62 (0.17)	2.94 (0.20)	+12.2	2.67 (0.13)	3.10 (0.24)	+16.1
Leaf, 1996b day	1.96 (0.06)	2.10 (0.14)	+7.1	1.77 (0.13)	2.09 (0.23)	+18.1
Leaf, 1996 night	1.68 (0.13)	1.69 (0.10)	+5.9	1.47 (0.17)	1.95 (0.31)	+32.6
Stem, 1996 day	4.53 (0.74)	3.82 (0.48)	−15.7	3.50 (0.50)	5.40 (0.41)	+54.3
$\bar{L}$ (%)‡			+9.0			+21.8
(95% CI)			(0–19)			(9–36)

Notes: Leaves were measured once in 1995 during the day, twice in 1996 during the day (1996a and 1996b), and once in 1996 during the night; stems were measured once in 1996 during the day. Respiration data are means with 1 SE in parentheses; n = 3–5 replicate chambers per CO_2 and soil-N availability treatment.
† Percentage change due to CO_2 treatment.
‡ The mean CO_2 effect size, $\bar{L}$, was calculated from the log-transformed ratios of elevated to ambient means within a soil N treatment (see *Methods: Statistical analysis* for details).

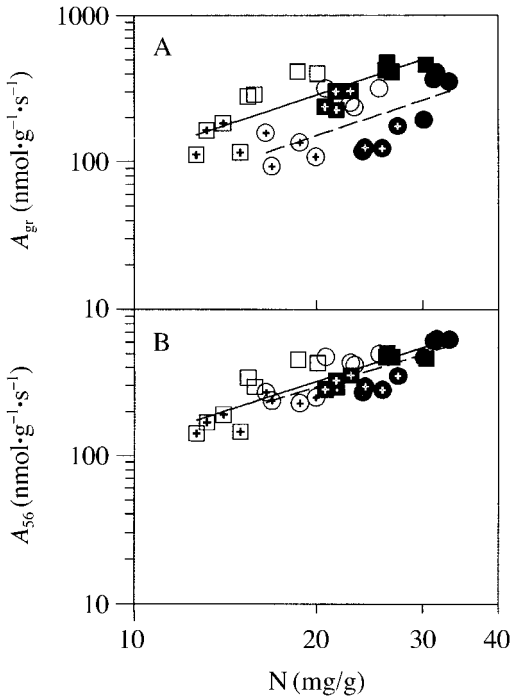

FIG. 1. Change in net CO_2 assimilation in relation to leaf N of *Populus tremuloides* growing in low-N (open symbols) or high-N (solid symbols) soil and at ambient (circles, dashed line) or elevated (squares, solid line) CO_2. Note log scales. Measurements of *A* (leaf-level light-saturated net CO_2 assimilation) were made in 1996 on day of year 157 (symbols without +) or day of year 197 (symbols with +) and both at growth p(CO_2) (A) and 56 Pa C_i (B). Each point represents the mean response of one chamber with lines fitted by least-squares regression.

at 56 Pa C_i. There was a significant difference in intercept for lines fitted to elevated- vs. ambient-CO_2 plants at growth p(CO_2) (Fig. 1A) but not at a common C_i of 56 Pa (Fig. 1B) or 27 Pa (data not shown). Note that tissue N concentration and *A* declined in all treatments between the day of year (DOY) 157 and DOY197 sampling dates.

Leaf tissue chemistry

Leaf N showed a consistent response to the treatments across the latter two years of the experiment (Table 5). There was a reduction in N_m (leaf N on a mass basis) due both to CO_2 enrichment and low soil-N availability, leading to increases in tissue C/N. For N_a (leaf N on an area basis), however, the relative effect of CO_2 was substantially less, becoming significant only in low-N soil. Leaf total non-structural carbohydrate (TNC) increased with elevated CO_2 and low-N soil, due primarily to increased starch concentration (Fig. 2). This increase, together with parallel treatment effects on leaf thickness led to significant reductions in specific leaf area (SLA) in elevated compared with ambient-CO_2-grown plants (Table 5). Changes in leaf TNC accounted for ~58% of the decrease in SLA in low-N soil and ~54% of the decrease in high-N soil.

Aboveground growth

Aboveground growth was modest in the first year of the experiment, as plants acclimated to the chamber environment. During the first year of growth, leaf area and stem volume increased in high-N soil compared to low-N soil but showed no response to CO_2 enrichment (Fig. 3, Table 6). During the second year, however, aboveground growth was dramatic, particularly in high-N soil where stem volume increased over 50-fold

TABLE 5. Leaf chemical and physical properties of *Populus tremuloides* grown in low- or high-N soil and at ambient or elevated CO_2.

Leaf property	Low-N soil				High-N soil			
	Ambient CO_2		Elevated CO_2		Ambient CO_2		Elevated CO_2	
	Mean	1 SE	Mean	1 SE	Mean	1 SE	Mean	1 SE
August 1995								
N_m (%)	2.1^{a}	0.1	1.6^{c}	0.1	2.7^{b}	0.1	2.1^{a}	0.1
N_a (g/m^2)	1.3^{c}	0.1	1.1^{b}	0.03	1.8^{a}	0.1	1.7^{a}	0.1
C (%)	50.5^{ae}	0.1	50.1^{c}	0.2	51.0^{bd}	0.1	50.3^{ace}	0.1
C/N	25.1^{a}	1.3	33.3^{c}	1.7	19.4^{b}	0.4	24.7^{a}	0.6
SLA (m^2/kg)	15.8^{d}	0.4	13.8^{ce}	0.7	14.9^{bcd}	0.3	12.0^{ac}	0.6
July 1996								
N_m (%)	1.8^{d}	0.1	1.4^{c}	0.1	2.5^{b}	0.1	2.2^{a}	0.04
N_a (g/m^2)	1.3^{c}	0.1	1.2^{b}	0.03	1.7^{a}	0.04	1.7^{a}	0.04
C (%)	50.8^{a}	0.1	50.6^{a}	0.3	50.8^{a}	0.3	50.4^{a}	0.1
C/N	29.3^{c}	1.3	38.3^{b}	1.7	20.6^{a}	0.8	23.8^{a}	0.6
SLA (m^2/kg)	13.5^{de}	0.1	12.1^{c}	0.3	14.6^{be}	0.2	12.8^{acd}	0.4
L_t (μm)	136^{ad}	3.1	146^{b}	1.7	128^{cd}	3.7	137^{ab}	2.9

Notes: Leaves were sampled for N, C, and specific leaf area (SLA) following gas-exchange measurements in 1995 and 1996 and for leaf thickness (L_t) prior to harvest in 1996. Leaf N is expressed on both a mass (N_m) and an area (N_a) basis; $n = 5$ replicate chambers per CO_2 and soil-N availability treatment. Within a row means with the same lowercase superscript letter are not significantly different at $P < 0.05$.

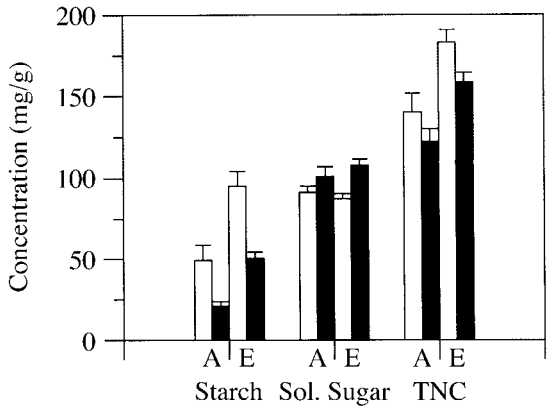

FIG. 2. The concentration of starch, soluble sugar (Sol. Sugar), and total non-structural carbohydrates (TNC) in *Populus tremuloides* leaves after 2.5 seasons of growth in low-N (open bars) or high-N (solid bars) soil and at ambient (A) or elevated (E) CO_2. Data are means and 1 SE; $n = 5$ replicate chambers per CO_2 and soil-N-availability treatment.

compared to a ~7-fold increase in low-N soil. Carbon dioxide enrichment resulted in a significant increase in both leaf area and stem volume in year 2, but only in high-N soil. In that soil treatment, the increase in stem volume was nearly equally distributed between second-year growth (+28%) and first-year growth (+35%). Second-year stem volume was over twice that of first-year stem volume in low-N soil but only 20–30% greater in high-N soil.

The CO_2 and soil-N-availability effects on growth observed in year two continued into year three (Fig. 3, Table 6). In high-N soil, elevated CO_2 increased both leaf area (+28%) and stem volume (+37%), but CO_2 had no effect in low-N soil. In high-N soil, all yearly growth classes showed increased stem volume at elevated compared to ambient CO_2. Wood density increased significantly in low- compared to high-N soil and due to CO_2 enrichment in first- and second-year wood in low-N soil (Table 6). Elevated CO_2 caused an increase in harvested stem mass in all yearly growth classes in high-N soil, resulting in a 34% increase in total aboveground wood production. In low-N soil, no year class responded significantly to CO_2 enrichment, and total wood production was consequently unaffected by CO_2 treatment.

This pattern of treatment response was reflected in the ANOVA of harvested leaf area and stem mass (Table 3). The effects of CO_2 and soil-N availability were highly significant, as was the interaction between atmospheric CO_2 and soil-N availability. The effect of genotype also was significant for these measures, as was the genotype × soil N interaction. For example, in low-N soil there was no difference in final leaf area among genotypes while in high-N soil genotype 1L had over twice the leaf area as genotypes 42E and 51E (Fig. 4). A similar response was observed for final stem volume and mass, and for stem volume and leaf area in year two (data not shown). At the final harvest, we found no genotype × CO_2 or genotype × CO_2 × soil N interaction, indicating a similar aboveground growth response to CO_2 by all genotypes in each soil N treatment.

The vertical distribution of leaf area in year three was not markedly affected by CO_2 treatment (Fig. 5). In high-N soil, CO_2 enrichment increased leaf area throughout the canopy, with a somewhat greater response in the upper third of the canopy compared with the lower sections. However, the relative distribution of leaf area among canopy-position classes was unchanged. There was no effect of CO_2 on leaf area in any canopy position in low-N soil.

Stem volume growth efficiency increased with increasing chamber leaf-area index (LAI) across the three years of the experiment (Fig. 6). Although final LAI was highest at elevated CO_2 and in high-N soil, and trees in high-N soil had over twice the leaf area as did those in low-N soil, we were unable to distinguish separate functional relationships between E_v (aboveground volume growth efficiency) and LAI for the different treatment combinations. That is, all treatments appeared to have similar E_v at equivalent LAI. Stem mass growth efficiency, obtained at the final harvest, showed

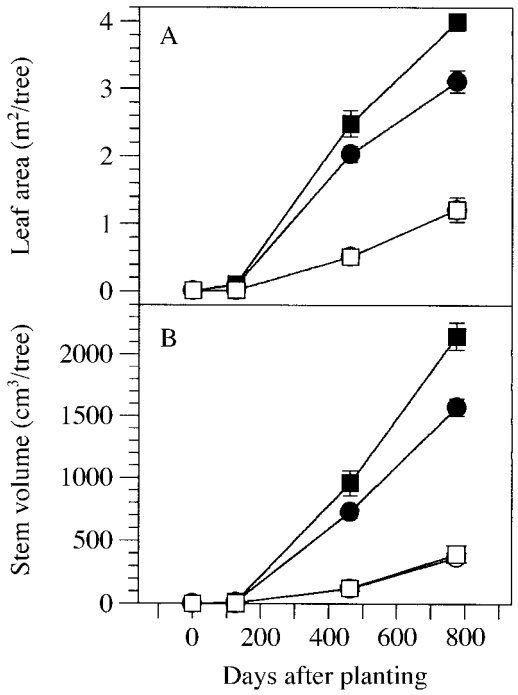

FIG. 3. Change in leaf area (A) and stem volume (B) of *Populus tremuloides* during 2.5 seasons of growth in low-N (open symbols) or high-N (solid symbols) soil and at ambient (circles) or elevated (squares) CO_2. (In some cases open circles are completely hidden from view by open squares.) Data are means ± 1 SE; $n = 5$ replicate chambers per CO_2 and soil-N-availability treatment.

TABLE 6. Stem volume (S_v), stem density (S_d), and stem dry mass (S_{dm}) per tree of *Populus tremuloides* grown in low- or high-N soil and at ambient or elevated CO_2.

Year	Stem parameter	Low-N soil				High-N soil			
		Ambient CO_2		Elevated CO_2		Ambient CO_2		Elevated CO_2	
		Mean	1 SE	Mean	1 SE	Mean	1 SE	Mean	1 SE
1994	Total S_v (cm^3)	1.4^b	0.2	1.8^b	0.6	12.8^a	3.9	16.2^a	3.0
1995	S_{v1} (cm^3)	35.7^b	4.4	41.4^b	9.7	318.6^a	42.4	431.0^a	62.0
	S_{v2} (cm^3)	83.7^c	8.9	84.3^c	17.3	411.5^b	29.3	527.5^a	41.4
	Total S_v (cm^3)	119.4^c	13.1	125.7^c	25.3	730.1^b	52.2	958.5^a	99.4
1996	S_{v1} (cm^3)	81.7^c	10.0	100.3^c	15.1	480.3^b	56.5	648.3^a	77.2
	S_{v2} (cm^3)	205.6^c	19.3	204.3^c	33.4	829.4^b	28.2	1094.9^a	44.2
	S_{v3} (cm^3)	82.2^c	10.6	92.2^c	21.5	265.7^b	22.8	400.2^a	32.3
	Total S_v (cm^3)	370.8^c	34.3	396.3^c	65.7	1575.4^b	70.3	2150.2^a	109.4
	S_{d1} (g/cm^3)	0.444^c	0.010	0.472^b	0.011	0.388^a	0.005	0.395^a	0.002
	S_{d2} (g/cm^3)	0.400^c	0.007	0.449^b	0.010	0.361^a	0.007	0.338^a	0.006
	S_{d3} (g/cm^3)	0.409^a	0.012	0.502^a	0.043	0.367^a	0.008	0.414^a	0.059
	S_{dm1} (g)	35.0^c	4.2	43.9^c	6.1	182.1^b	19.2	250.6^a	31.2
	S_{dm2} (g)	78.6^c	8.1	82.6^c	11.1	282.8^b	5.6	356.4^a	14.2
	S_{dm3} (g)	32.0^c	3.6	37.5^c	7.7	93.0^b	7.8	139.4^a	9.3
	Total S_{dm} (g)	145.6^c	13.9	164.1^c	22.2	557.9^b	22.4	746.5^a	47.7

Notes: In 1994 and 1995, S_v was estimated from non-destructive branch measurements and in 1996 from harvest data. In 1995 and 1996, measurements were further subdivided according to the year class of growth (subscript 1 = year class 1, etc.); n = 5 replicate chambers per CO_2 and soil-N availability treatment. Within a row, means with the same lowercase superscript letter are not significantly different at $P < 0.05$.

a similar pattern, with no significant CO_2 effects on E_{dm} within soil N treatments (Fig. 6). Similar results were obtained when all trees, not just the central individuals in each chamber, were included in the analysis (data not shown).

DISCUSSION

Elevated-CO_2 effects on A (leaf-level light-saturated net CO_2 assimilation) in our study followed a pattern typical of many woody species, with multi-year increases in A_{gr} (A at growth CO_2 partial pressure) (Norby et al. 1992, Tissue et al. 1993, Eamus et al. 1995) but also variation in the magnitude of this response temporally (Gunderson et al. 1993, Lewis et al. 1996) and with soil fertility (Tissue et al. 1993). In high-N soil, we found little evidence for A_{accl} (acclimation of A at high CO_2) in elevated-CO_2-grown plants, similar to the results of Curtis et al. (1995), Will and Ceulemans (1997), and Kalina and Ceulemans (1997), all working with hybrid poplar. In low-N soil, however, A_{accl} was apparent by the second year of our study and had increased in magnitude by the third year. The physiological and environmental factors leading to A_{accl} at high CO_2 have been the focus of much experimental and theoretical work (Sage 1994, Lloyd and Farquhar 1996). One factor consistently associated with A_{accl} in both woody and herbaceous species is a decline in leaf tissue N concentration (McGuire et al. 1995, Peterson et al. 1999), although not all plants with lower leaf N at high CO_2 show strong A_{accl} (e.g., Will and Ceulemans 1997). It is important in that regard to distinguish between a net reduction in N per unit leaf mass or leaf area that is attributable to a reduction in N content, and a reduction in N concentration due to the dilution effect of leaf starch accumulation without a change in N content. Reduced Rubisco (Ribulose bis-phosphate carboxylase-oxygenase) content is generally the assumed consequence of lower leaf N and is considered a primary physiological mechanism by which A_{accl} occurs (Stitt 1991). However, plants may compensate for lower leaf-N concentration (mass basis) with additional mesophyll cells, thereby maintaining or even increasing photosynthetic capacity per unit leaf area (Luo et al. 1994).

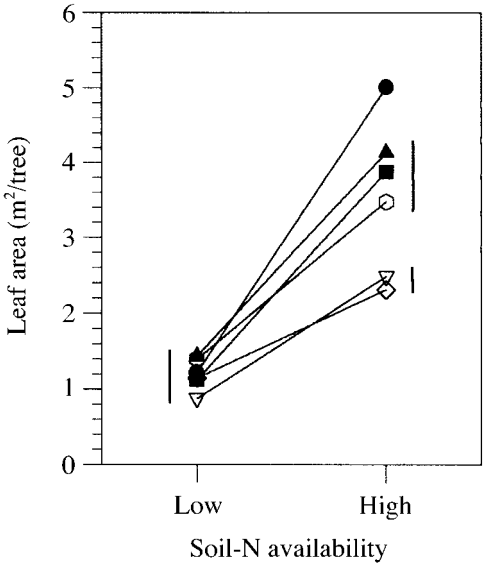

FIG. 4. Genotypic differences in leaf-area growth in soils of differing N availability in *Populus tremuloides*. Genotypes are distinguished by different symbols and whether they are of early (open symbols) or late (solid symbols) leaf-drop phenotype. Vertical bars indicate groups of means that are not significantly different from one another at $P < 0.05$.

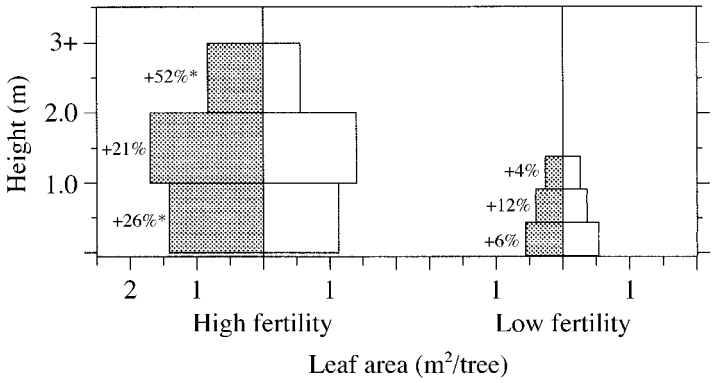

FIG. 5. Vertical distribution of leaf area in *Populus tremuloides* after 2.5 seasons of growth in soil of low or high N availability and at ambient (open bars) or elevated (shaded bars) CO_2. The percentage increase due to CO_2 is indicated to the left of the shaded bars (* $P < 0.05$).

Our results support the model of Luo et al. (1994) identifying reciprocal changes in N_m (leaf N on a mass basis) and SLA (specific leaf area) as important controls over the direction and magnitude of CO_2 effects on *A*. In high-N soil, the modest decline in N_m is entirely compensated for by increasing leaf thickness and lower SLA, with the result that N_a (leaf N on an area basis) remains unchanged in elevated- compared to ambient-CO_2-grown plants, and there is no A_{accl}. With a greater reduction in N_m as seen on low-N soil, there was only partial compensation by lower SLA in CO_2-enriched plants and therefore N_a declined, leading to significant A_{accl}. Our data also suggest that the fundamental *A*:N relationship, reflecting leaf-level allocation of N into different photosynthetic and non-photosynthetic constituents, was unchanged by either CO_2 or soil-N treatments. That is, at a common C_i (leaf internal CO_2 partial pressure) of 56 Pa, all treatments fell along a common line relating log(A_m) (*A* on a leaf mass basis) to log(N_m), and whose slope (1.31) was similar to that reported by Reich et al. (1997) for a collection of 111 species across six biomes (1.41). The effect of CO_2 enrichment was to change the intercept of that line, but not its slope. This is equivalent to an increase in photosynthetic nitrogen-use efficiency (Peterson et al. 1999). At equal leaf N_m, high-CO_2-grown plants in our study would have a 93% greater A_m. The acquisition of soil N and its allocation within the plant, both temporally and spatially, is thus clearly of central importance in understanding the long-term consequences of rising CO_2 on photosynthesis in *Populus tremuloides.*

Aboveground biomass accumulation in *P. tremuloides* after 2.5-growing-seasons exposure to elevated CO_2 in soils of contrasting N availability was very similar to the average response of 21 woody-plant species to similar treatments as estimated in the meta-analysis of Curtis and Wang (1998). This was true both in the absolute magnitude of the CO_2 response and in the relative effects of low N availability. We found a 34% increase in stem dry mass at twice-ambient CO_2 and in high-N soil, compared to a 39% increase reported by Curtis and Wang (1998). Low soil-N availability reduced the CO_2 effect on leaf-area growth to near zero and reduced the stem dry mass responses by ~60%, both common although not universal responses among woody plants (Lloyd and Farquhar 1996). Whole-plant biomass mirrored these responses (+37% at high N, +17% at low N, Zak et al. 2000*a*) and were again very close to the average values for woody plants estimated by Curtis and Wang (1998). A complete account of CO_2 effects on biomass partitioning in this experiment can be found in the companion paper by Zak et al. (2000*a*).

Leaf-area distribution within the canopy can have important effects on light interception and growth in *Populus* and can be used in selection programs for rapidly growing genotypes in short-rotation forestry (Ceulemans et al. 1990). The sylleptic growth of lateral branches characteristic of this genus also results in a high degree of architectural plasticity in response to environmental factors such as planting density (Nelson et al. 1981) or soil fertility (Heilman and Xie 1994). In a CO_2-enrichment study with six tropical tree species of indeterminate growth habit, Reekie and Bazzaz (1989) found that mean canopy height increased at high CO_2 in two species, decreased in one species, and was unchanged in three others. They concluded that CO_2 effects on canopy structure played a major role in competitive interactions among these species. We found little evidence for changes in the vertical distribution of LA (total leaf area per plant) due to growth at high CO_2. In high-N soil, the majority of LA was in the middle stratum, regardless of CO_2 treatment and consistent with the architecture of *Populus* canopies when grown under similar planting densities (Ceulemans et al. 1990, Kubiske et al. 1997). However, elevated CO_2 caused a greater increase in LA in the upper canopy (+52%) than in the middle (+21%) or lower (+26%) strata, an effect that over time would result in increased self-shading within the canopy and a decline in canopy growth efficiency. Plants grown in low-N soil had much lower total LA relative to plants in high-N soil and the greatest fraction of canopy LA was in the lowest stratum. Again, exposure to high CO_2 did not change this pattern of LA distribution. It should be noted that although our removal of terminal shoot growth during

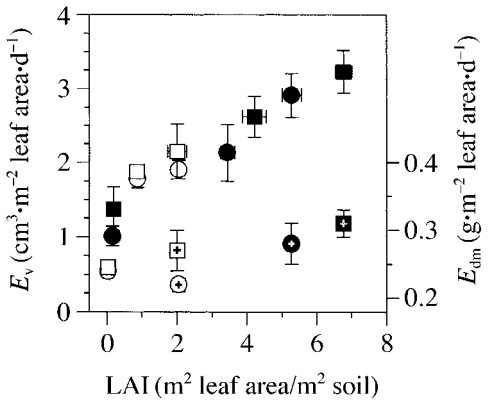

FIG. 6. Aboveground growth efficiency (E; growth efficiency, wood production per unit leaf area per unit time) in relation to chamber leaf-area index (LAI) of *Populus tremuloides* grown in low-N (open symbols) or high-N (solid symbols) soil and at ambient (circles) or elevated (squares) CO_2. Growth efficiency was estimated for plants growing in the center of each chamber on the basis of annual stem volume increment (E_v, symbols without +) or harvested stem mass (E_{dm}, symbols with +). Data are means ± 1 SE; $n = 5$ replicate chambers per CO_2 and soil-N-availability treatment.

the second winter likely resulted in increased lateral branch production and relatively greater LA lower in the canopy, these effects should be independent of either CO_2 or soil N treatments since all plants were similarly treated.

Populus tremuloides shows substantial genotypic variation in a number of growth and physiological characteristics, including trunk morphology, lateral-branch abscission, timing of leaf drop (Barnes 1959), and sensitivity to ozone stress (Karnosky et al. 1989). Indeed, the large amount of genetic variation present in this, and other, members of the genus *Populus* has provided ample opportunity for breeding and improvements in biomass yield (Farmer 1996). We also observed significant genotypic variation in growth characteristics and in responses to changing soil-N availability. However, we found no genotype × CO_2 interactions, indicating a uniform growth response to CO_2 enrichment by all six genotypes in our study. This is perhaps not surprising given the small number of genotypes examined and the relatively short duration of the experiment. We have found CO_2 × genotype interactions in these same genotypes for condensed tannin production (Mansfield et al. 1999) and early season photosynthetic rates (X. Wang, *unpublished data*), both of which might eventually influence biomass accumulation. There has been little other work conducted on intraspecific variation in CO_2 responses within woody plant species. Radaglou and Jarvis (1990) reported variation in leaf structural characteristics at high CO_2 among hybrid poplar genotypes, while Kalina and Ceulemans (1997) found greater A_{accl} at high CO_2 in a slow-growing poplar genotype compared to a fast-growing genotype. Rising atmospheric CO_2 clearly has the potential to act as a selective agent (Curtis et al. 1996) and hence alter both ecosystem composition and function. Identification of traits that are responsive to CO_2 enrichment and that contribute directly to growth and fitness should receive continued attention by forest geneticists and evolutionary biologists.

Whole-plant net C gain is a function of the assimilatory capacity of leaves, the area and arrangement in space and time of leaf surface, and the loss of carbon through respiration, tissue abscission, and processes such as rhizodeposition and volatile organic-carbon emissions. Elevated CO_2 has the potential to affect each of these processes, and it is therefore not surprising that there is no simple relationship between, for example, CO_2 effects on A and CO_2 effects on harvested biomass. Measures that integrate assimilatory and allocational processes over time, such as those obtained through growth analysis, may be more useful in interpreting and predicting environmental effects on production than are short-term physiological measures such as A. In forests, as with herbaceous crops, productivity is strongly related to total light interception, and stand growth, or yield, may be estimated by a knowledge of maximum canopy leaf-area index (LAI) and growth efficiency (E, wood production per unit leaf area per unit time) (Waring 1983). As stand development proceeds, E typically declines due to increased competition for light and allocation to non-photosynthetic tissue (Cannell 1989). However, productivity will continue to increase as long as LAI increases faster than E decreases. Heilman and Xie (1994) found a linear relationship between stand productivity and LAI in poplar hybrids up to an LAI of ~6, with a declining rate of increase at higher LAI.

Growth efficiency was low in our experiment during the first year of growth, likely due to the energy costs of root establishment following outplanting from cuttings. Although we could not measure total belowground biomass prior to harvest, a decline in root-to-shoot ratio with plant age, as is typical in young *Populus* plants (Sheppard and Smith 1993), would result in increasing E with time, as was observed in our study. This trend would be opposed by increasing competition for light as LAI increased and by greater sapwood respiratory demand with increasing stem volume. Neither soil-N availability nor CO_2 enrichment had any apparent effect on the relationship between LAI and E_v, however. That is, while at any point in time plants in high-N soil were larger than plants in low-N soil, and CO_2 enrichment increased plant size in high-N soil, at equivalent stages of canopy development all treatments produced stem volume with equal efficiency. The basic allometric relationship between LA and stem volume (or mass) increment was unaltered by CO_2 enrichment, and we would therefore expect no change in aboveground stand wood volume or mass at equivalent LAIs, at least through an LAI of ~6. These results support

others showing little effect of elevated CO_2 on woody-plant allometry (Gebauer et al. 1996, Zak et al. 2000*a*).

However, important questions remain. In particular, we do not know whether *E* will remain equivalent between CO_2 treatments as LAI increases. If quantum yield is increased by high CO_2, as suggested by Long and Drake (1991), we would expect *E* to be sustained as LAI increases to a greater extent in elevated- compared to ambient-CO_2-grown plants due to reduced self-shading effects on carbon assimilation. We also do not know maximum LAI for any of the treatment combinations, another important determinant of long-term stand productivity. For example, maximum LAI in low-N soil or at ambient CO_2 may never reach that obtained in high-N soil or at elevated CO_2. Answers to these questions require experiments at temporal and spatial scales beyond those achievable with open-top chambers.

These results illustrate several important issues regarding tree growth at high CO_2. The first is that increased A_{gr} does not necessarily lead to increased *E*. For *P. tremuloides* in high-N soil, higher A_{gr} due to CO_2 enrichment was offset by higher rates of carbon loss, via increased leaf and stem respiration and from greater fine-root turnover (Pregitzer et al. 2000). In effect, increased stem-biomass accumulation could be accounted for entirely by greater LA. In low-N soil, CO_2 stimulation of A_{gr} was less, and varied between years, but also did not translate into significantly greater *E*. The second issue is that knowledge of the dynamic response of *E* with respect to LAI will be necessary to answer the question of whether trees will grow larger or simply grow faster during stand development (Eamus and Jarvis 1989). Our data suggest the latter. That is, in high-N soil stand productivity increased at high CO_2, but the fundamental relationship between tree growth and canopy development appeared unchanged (see also Zak et al. 2000*a*).

Summary and implications

Elevated atmospheric CO_2 had a sustained, positive effect on carbon-gain capacity and aboveground biomass accumulation in *P. tremuloides*, but only under conditions of high soil-N availability. Carbon dioxide enrichment increased photosynthetic N-use efficiency regardless of soil-N availability but in low-N soil growth at elevated CO_2 resulted in lower leaf-N concentration. This led to a reduction in photosynthetic capacity relative to plants at ambient CO_2. Gains in net CO_2-assimilation rate under elevated CO_2 were largely offset by increased dark respiration and fine-root turnover, resulting in no net increase in growth efficiency at high CO_2. Nonetheless, increased stand productivity with elevated CO_2 and high soil-N availability could shorten harvest rotation intervals for this economically important species and speed ecological succession in aggrading northern hardwood forests.

Acknowledgments

We thank Diana Randlett and Bill Holmes for help in experimental design and execution, Mark Kalnins for leaf anatomical work, and Richard Norby for comments on an earlier draft. This work was funded through grants from the National Institute for Global Environmental Change (DOE-NIGEC), Program for Ecosystem Research (DOE-PER Grant DE-FGOZ-93ER6166), the University of Michigan Project for the Integrated Study of Global Change, and the University of Michigan Biological Station.

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